佐大農彙　46：1～8（1979）

Studies on the Growth of Root Hairs in Rice Plants and Their Microfibrous Network Structure with a Scanning Electron Microscope

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Received October 9, 1978

Introduction

Although it is well known that there occurs a multitude of fine white hairs radiating from all sides of the roots of crop plants, there are many unclear respects in regard to the growth of root hairs, the condition of their surface, and the fibrous structure of the epidermal cell wall. However, recent development of freeze-drying[1,2,5] and critical point drying[7] techniques has made easy to observe the superficial condition of soft plant tissues in their native state.

The experiment was carried out to examine firstly the superficial conditions of a primary root and a root hair, and secondly the microfibrous structure of a root hair, with a scanning electron microscope and with the new drying methods. Moreover, the root of the peanut plant, which has been considered as root hairs are not to be appeared, was observed for reference.

Materials and Methods

The materials used for the study were root hairs of main roots of a rice plant (var. Reiho) and roots of a peanut plant (var. Chibahandachi).

In order to promote the growth of root hairs, seeds were sowed on three sheets of filter paper saturated with distilled water in a dish (14 cm in diameter, 1.5 cm in depth).

After sowing, the lid was put on the dish and the dish was kept at 28°C for about a week. When a root elongated about 2 cm and root hairs grew, the main root was cut off at its base for observation. The superficial conditions of a main root and a root hair were mainly observed with freeze-drying method, and fibrous structure of an epidermal cell wall was observed with critical point drying method.

Freeze-drying: After the sample mounted on the specimen holder, it was frozen with liquid nitrogen and the frost covering its surface was sublimated in the pre-evacuation chamber in cryostat. Then the sample prepared, but not dehydrated or metal coated, was quickly thrown into the chamber to observe directly under a scanning electron microscope incorporated with specially designed cold devices. A cold stage was installed in the specimen holder at temperature down to −150°C during most observation. The scanning electron microscope used was JSM–50A and accelerating voltage was 5 KV.

Critical point drying: The sample was firstly fixed in 1% glutal aldehyde at 4°C for twelve hours, then fixed in 2% osmium acid at 0°C for one hour. The water was replaced by taking it away through a series of increasing alcohol concentrations in the standard manner. In a similar way, the alcohol was replaced by amylacetate. The specimen in amylacetate was dried by critical point drying replacing the amylacetate by liquid carbondioxide at 35°C.

For observation of the fibrous structure of a cell wall of a root hair, after the sample was submerged successively into 2% NaOH and H_2SO_4 for 30 minutes each, then it was rinsed to remove the pectin compounds covering the fibrous network by ultrasomic waves (28KC) in 0.1 M phosphorous buffer solution. Preparations were coated under with a 200–300 Å thick film of C–Au while being rotated at a continually varying angle to the electrode. Specimens were examined with JSM–50A scanning electron microscope, operating at accelerating voltages of 10 KV.

Result

1. Root cap and Mucigel

The root cap was covered with thick mucigel sheath[6] up to about 300 μ from the tip, and cells detached from the root cap were observed within it (Fig. 1, 2). The thickness of the mucigel was decreased at about 500 μ from the tip, then cells of the root cap were exposed around the epidermis.

A cell of the root cap was cylindrical (30–70 μ in length, and 7–13 μ in diameter) although a little swelled during its preparations.

2. Development of Root Hairs

The papilla which is the initial symptom of a root hair can be observed in the range 800–1000 μ from the root tip (Fig. 2, 3). It seemed that the formation of papillae began to originate around the upper end of the elongation zone.

In this zone originating the papillae, two kinds of epidermal cells, long cells (100–120 μ in length) and short cells (60–80 μ), had already been divided and papillae were usually formed from the short cells with polarity. That is, the root hairs were produced not towards the basal end but towards the apical end (Fig. 3, 4).

The length of the root hairs were longer as the distance was away from the root tip, and its maximum length was 600 μ, 5–8 μ in maximum diameter at the 2.5 cm position from the tip (Fig. 5). The elongating direction of the root hair was usually at right angles to the root axis (Fig. 6).

The surface of the root hair was entirely smooth although some mucilagenous particles and bacteria adhered embedded on it (Fig. 7, 8). On the other hand, in peanut plants the root hairs were not formed from the epidermal cells, and numerous bacteria embedded on the surface of the epidermal cell wall as the rice roots (Fig. 9–12).

3. Microfibrous network structure

The microfibrous network structure can be observed under the superficial pectin layer (Fig. 13–19). A fine network of microfibers developed longwise and crosswise from the base to the tip in a young root hair or even in the papilla (Fig. 18, 19). The microfibrous network structure of the epidermal cell wall stretched into the wall of the root hair (Fig. 16), and the microfibrous network of the base was finer than that of the tip. The diameter of the microfiber was about 0.02 μ (Fig. 17).

Discussion

Mori (1972)[4] suggested "epidermal cells may be advanced in their vacuolation or maturation, and some may start growing root hairs after the elongation of the epidermis ceases". Kawata et al. (1957)[3] recognized "the root hairs in the moist chamber were

produced in the range of 1000–1200 μ away from the root tip (except root cap) in the seminal root, but they were produced at 900–1000 μ from the tip in the crown roots". The author, however, found that the papillae were produced at about 600 μ from the tip. This position was closer to the root tip than that Mori and Kawata et al. indicated. This suggested that the papillae began previously to be produced in the elongation zone.

As to the epidermal cell which produced the root hair, Kawata et al. said that it may be produced in the short cell and not in the long cell. The author also found the same phenomenon as Kawata et al.

Moreover, the originating position of papillae was not towards the basal end but towards the apical end in the epidermal cell as pointed out by Kawata and Ishihara.[3)]

It was also clear that the fibrous network structure of the root hair wall developed already in the papilla stage. The surface of the root hair was entirely smooth but numerous bacteria were embedded on it.

In peanut, no root hairs were produced but bacteria were embedded on the epidermis as were seen on rice roots and root hairs.

Summary

The superficial condition of a primary root of rice plant, the development of root hairs and the microfibrous network structure of their cell walls were clarified with a scanning electron microscope.

It has been so far extremely difficult to get sight of such a soft plant tissue as a root hair in its native state under the scanning electron microscope, so that the author in this experiment introduced freeze-drying and critical point drying techniques and tried to observed a root hair directly.

The results obtained were as follows:

1) Root hairs were produced from the epidermal cells as the papillae at the range between 500 nm and 700 nm from the tip. At this position, two kinds of cells different in length, long and short, had already been differentiated among the epidermal cells, and papillae were usually formed in the shorter cells.

2) The root cap and a part of a primary root were covered with mucigel sheath up to 200 nm from the root tip and detached cells of the root cap were observed adherent therein.

3) The polarity was recognized in regard to the position where the papilla was formed in the epidermal cell, that is, the papilla was entirely produced not towards the basal end but towards the apical end of the epidermal cell.

4) The surface of the root hair was overall smooth although some mucilaginous particles were found thereon. On the other hand, there could be observed numerous bacteria, which seemed to embed therein rather than adhere thereon.

5) A microfibrous network structure of a cell wall of a root hair developed longwise and crosswise under the superficial pectin layer, and the network was finer at the base than at the apex. The size of a microfiber was 200–300 Å in diameter.

6) An interconnection can be observed between the fibrous network structure of a root hair and that of an epidermal cell.

7) In peanut, root hairs did not develop from the epidermis, but numerous bacteria were observed embedded on the epidermal cell wall.

References

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Explanation of Figures

Fig. 1. A primary root of *Oryza sativa* L.. Root hairs were observed as papillae at about 700 μ from the tip.
p: papilla m: mucigel sheath ×25

Fig. 2. Enlargement of the root cap in Fig. 1. The surface of the root tip was covered by a mucigel sheath. The cells detached from the root cap were exposed around the epidermis. ×75

Fig. 3. Papillae, the initial symptoms of the root hairs, were observed in the short cells not in the long cells.
s: short cell l: long cell ×1000

Fig. 4. Enlargement of the part of the 'p'. Papillae usually originated at the apical end of the short cells with polarity. ×3000

Fig. 5. Various growth stages of the root hairs were observed in the root hair zone. ×75

Fig. 6. Enlargement of the part of the 'rh'. Root hairs usually elongated at right angles to the root axis. ×250

Fig. 7. The tip of a root hair. The surface of the root hair was entirely smooth but some small glanules of the mucigel and bacteria (b) were adhered on it.

Fig. 8. Enlargement of the tip of the root hair in Fig. 7. ×1500

Fig. 9. The surface of the epidermal cells of peanut plant. No root hairs developed from the epidermal cells. ×75

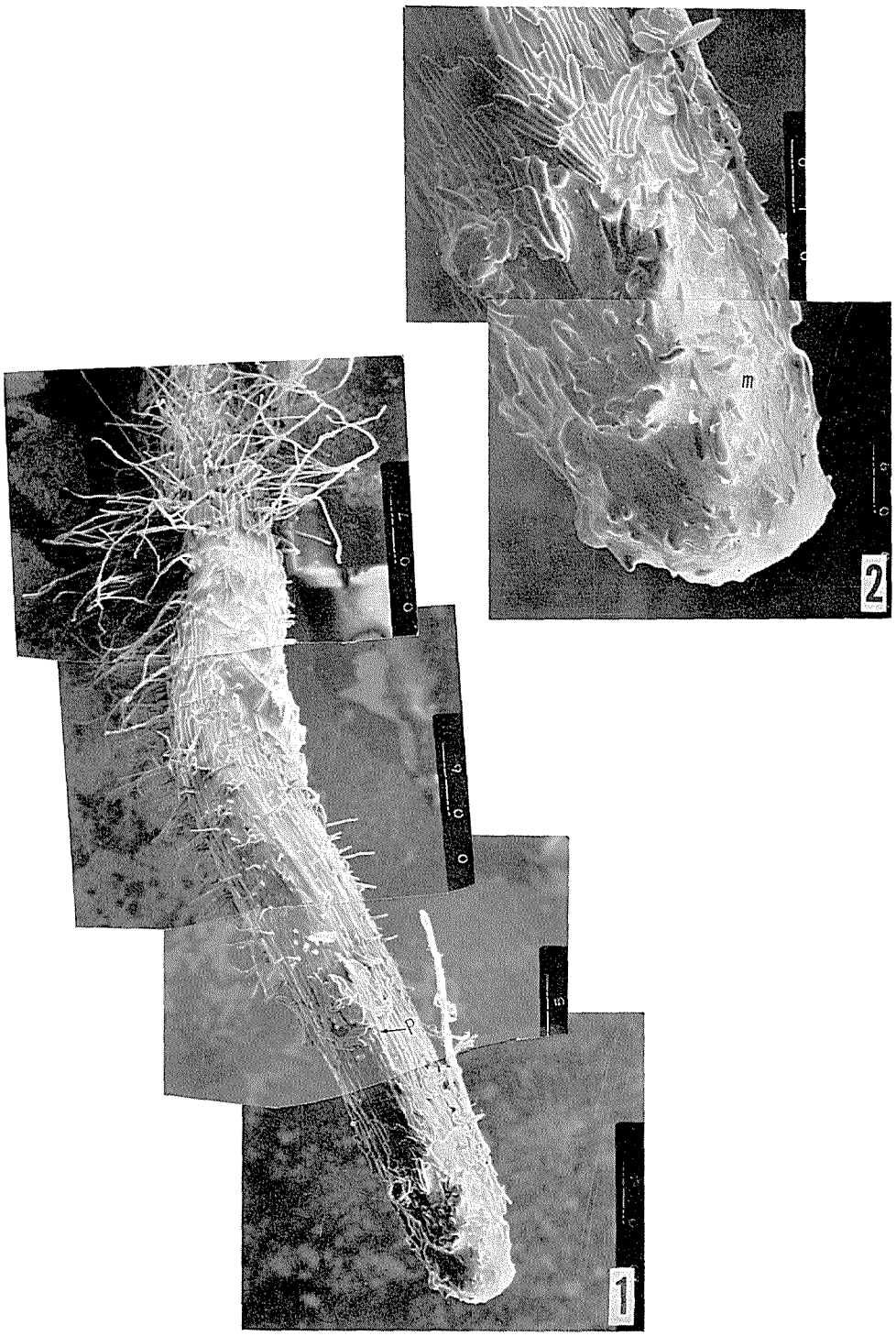
Fig. 10–12. Bacteria adhered on the surface of the root hair, they seemed to embedded therein rather than adhered thereon. ×750 (Fig. 10), ×250 (Fig. 11), ×750 (Fig. 12)

Fig. 13–15. A fine microfibrous network structure developed longwise and crosswise from the base extends to the tip of the root hair, and it was finer at the base than at the tip. Fig. 13 (tip), Fig. 14 (middle) and Fig. 15 (base). ×7500

Fig. 16. The fibrous network structure of the epidermal cell wall (e) stretched to the cell wall of the root hair (rh). ×15000

Fig. 17. Enlargement of the part of the 'e' in Fig. 16.
e: epidermis, rh: root hair ×25000

Fig. 18–19. The microfibrous network structure of the root hair was observed even at the papilla stage. ×1000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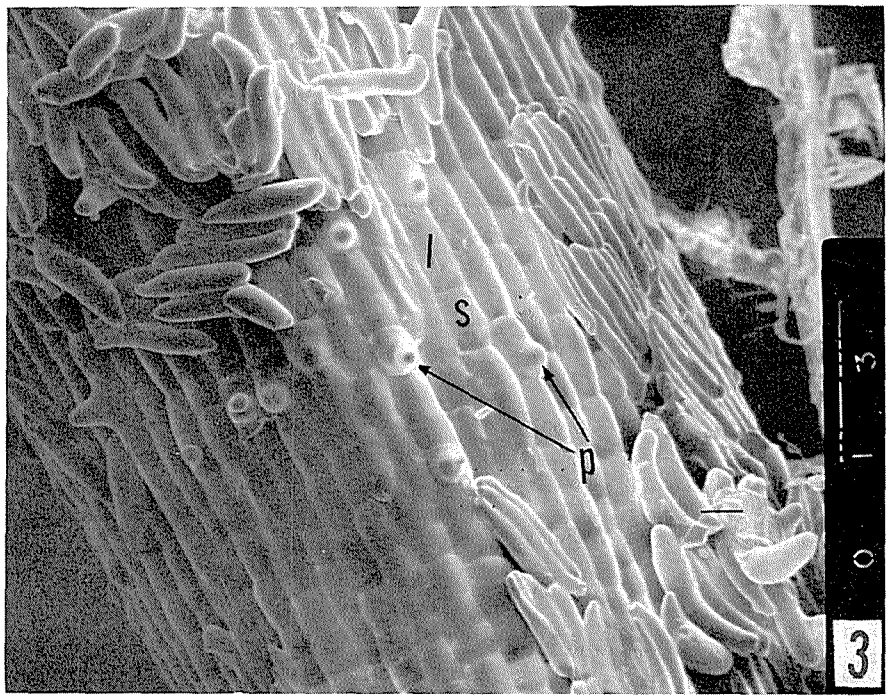
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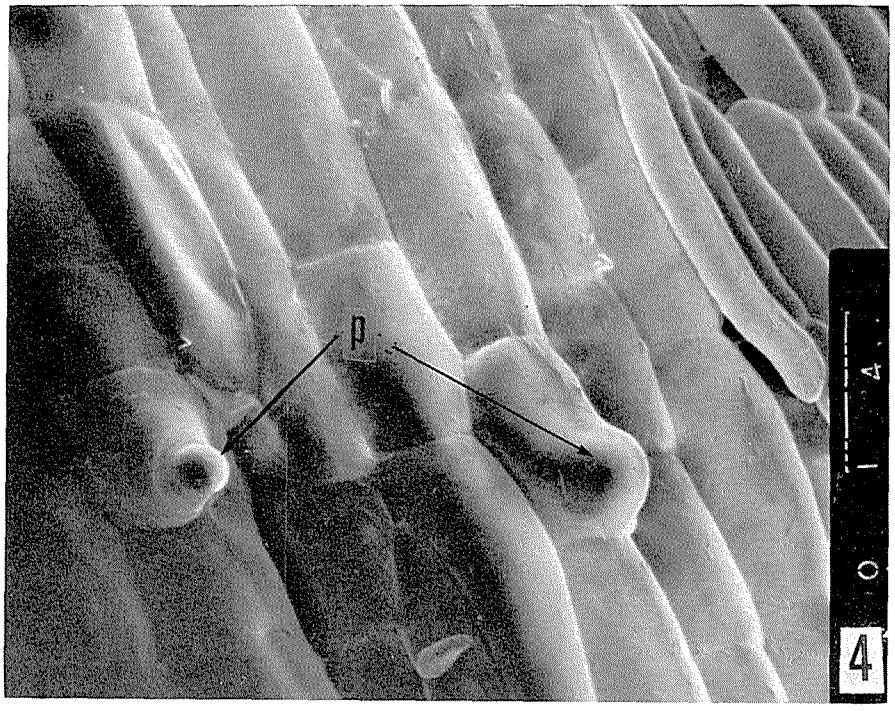
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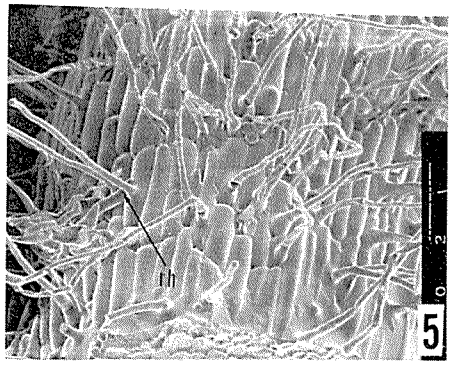
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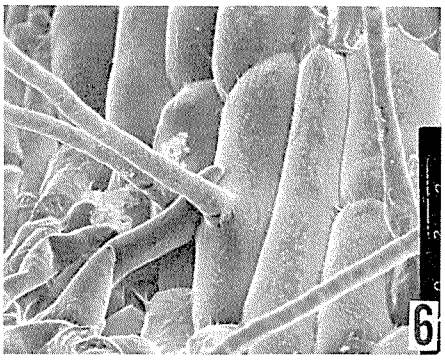
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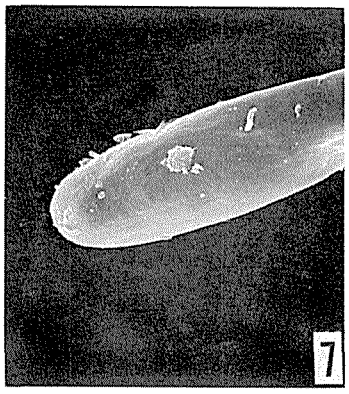
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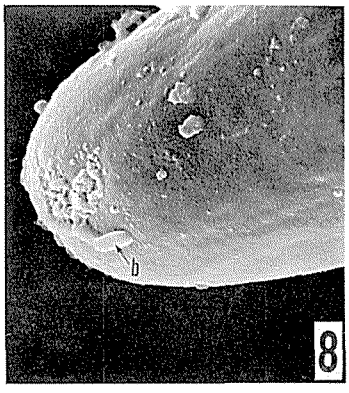
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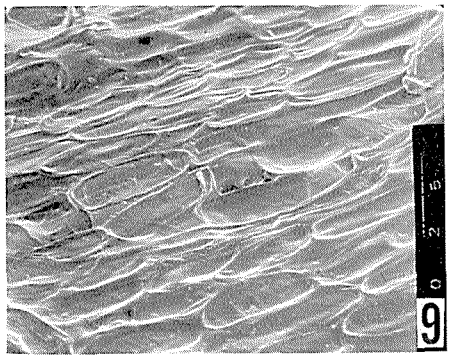
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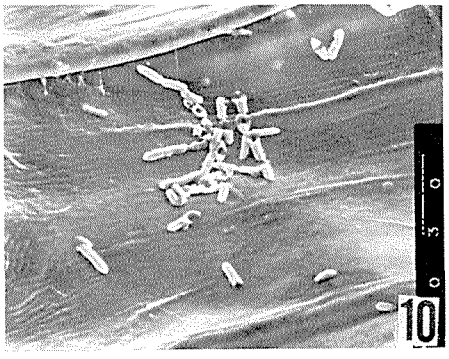
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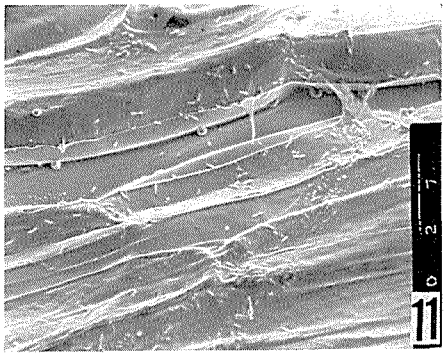
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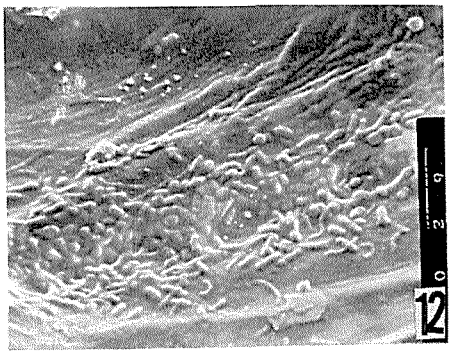
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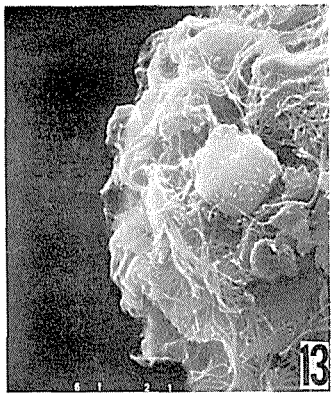

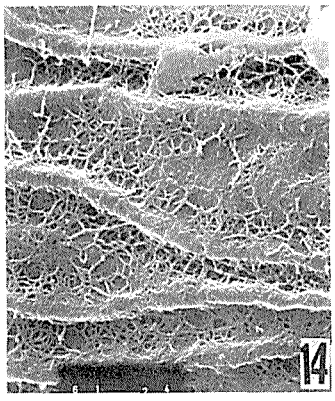

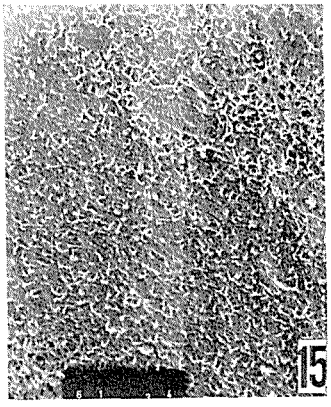

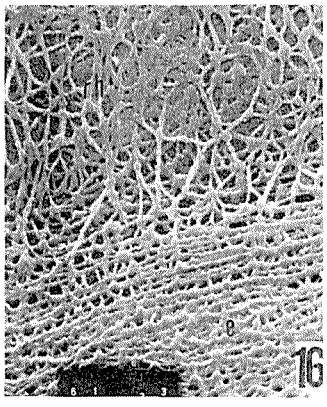

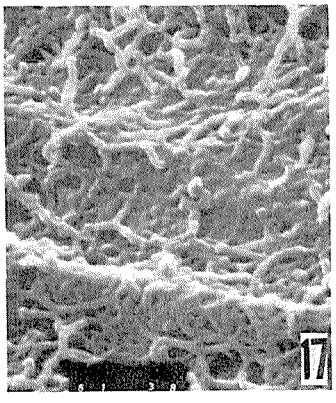

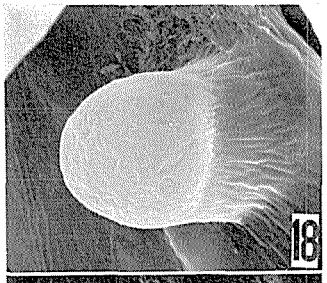

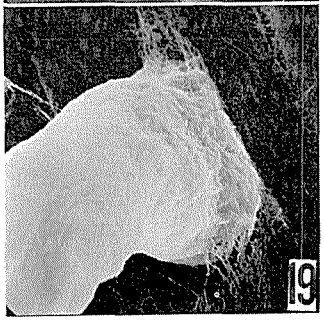

摘　　要

水稲根毛の形成に関する研究

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昭和53年10月9日　受理

走査顕微鏡を用い，凍結法および臨界点乾燥法によって，根毛表面の形状ならびに根毛壁内の微繊維網状構造を明らかにした。根毛は根端から 500～700μ の領域で表皮細胞から乳頭状突起として形成された。この部位には長，短2種の表皮細胞が分化しており，根毛は一般に短い細胞の根端寄りに形成された。根毛細胞壁には直径 200～300Å の微繊維からなる網状構造が認められた。根毛の表面は平滑であったが，おびただしい数のバクテリヤの着床が観察された。